EVALUATING CONNECTIVITY OF THE GULF COAST TICK POPULATIONS USING A POPULATION GRAPHICAL APPROACH

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Abstract

The Gulf Coast tick (GCT), *Amblyomma maculatum*, is undergoing range expansion. GCTs are the primary vectors of the pathogenic bacterium *Rickettsia parkeri* in North America. Prevalence of *R. parkeri* in GCT appears to be geographically structured such that ticks in populations outside of the historical range may pose disproportionately greater risks of human exposure to *R. parkeri* than ticks along the Gulf Coast. We hypothesized that mapping gene flow between GCT populations could reveal tick dispersal patterns, which might be driving higher *R. parkeri* prevalence in mid-Atlantic populations. We examined GCT population connectivity by inferring gene flow between using a Population Graph approach to landscape genetics. The Population Graph calculates covariances between populations based on the genetic relationships of individuals within and between populations. Ticks were collected in Virginia and Louisiana between 2017-2019. A total of 280 individual ticks haplotypes were included in the Population Graph from 6 populations. Haplotypes were sequenced using a fragment of the mitochondrial 16S rRNA gene in ticks. No isolation or cycling was identified in any population or subgroups. Further, a test of conditional Graph Distance (cGD) revealed no significant isolation by distance. We conclude that these GCT populations are generally panmictic.

Introduction

The burden of tick-borne diseases in the United States is a serious and increasing threat to public health (Sonenshine 2018). Understanding the ecology of TBD, particularly the environmental drivers that can influence host and tick population dynamics, thus influencing TBD spread, is an important complement to clinical work and biomedical research focusing on the pathogen. Population dynamics and range expansions of ticks are especially critical to understand if we intend to manage and control TBD. Models that forecast mosquito-borne illness risks (e.g. Barker 2019) offer examples of remote-sensing applications and the use of geospatial tools to address problems in the study of vector-borne disease. A similar approach that incorporates environmental data with tick and pathogen surveillance can be useful to understand TBD risks, with some modifications to use methods and spatio-temporal scales appropriate for ticks.

The Gulf Coast tick, *Amblyomma maculatum*, is a vector of the pathogen *Rickettsia parkeri*, the causative agent of a disease called *R. parkeri* rickettsiosis in humans (Paddock et al. 2004). Spotted fever rickettsiosis (SFR) is a diagnostic term that refers to disease caused by several *Rickettsia* spp. pathogens. *Rickettsia parkeri* causes a less severe disease than Rocky Mountain Spotted Fever (RMSF), caused by *R. rickettsii* (Paddock et al. 2008). Overall SFR's have a case fatality rate of between 5-10% (CDC, 2019). Incidence of SFR has

been increasing overall with higher incidences in states that lie in the southeastern and central United States. The regions with high human burden of SFR generally overlaps with the *A. maculatum* range in many areas, an ecological indication that *A. maculatum* are currently contributing to the disease burden in the region and may further amplify disease risk in the future. In fact, cases of *R. parkeri* rickettsiosis identified outside of the documented *A. maculatum* range in 2014 (Herrick et al. 2016) prompted further investigation and ultimately new collection records for *Amblyomma maculatum* ticks from Arizona and New Mexico (Allerdice et al. 2017; Hecht et al. 2020). These additional collections strengthen the connection between the emergence of *R. parkeri* rickettsiosis and the presence of *A. maculatum* ticks.

Amblyomma maculatum populations are expanding north from the species' historic range about 100-240 km from the Gulf Coast (Teel et al. 2010). This trend of range expansion has been accompanied by remarkable disparities in the geographic pattern of *R. parkeri* prevalence in the ticks collected in newly established populations along the northeastern margins of this range, specifically in the mid-Atlantic region (Wright et al. 2011, Paddock and Goddard 2015). Although it is not clear this pattern will continue, it has been consistently observed since 2010 (Wright et al. 2011, Nadolny et al. 2014). This prompts the question, what is the influence of geography on this vector-pathogen system? This question must be answered for each part of the system: the animal host, the tick vector, and the pathogen. Although these components are interconnected, the first step to understanding this system comprehensively is to understand the effects of geography on each part independently.

This paper explores the use of the Population Graph technique used in landscape genetics (Dyer and Nason 2004) to create a Population Graph. Population Graphs are useful for structuring genetic data in a way that can then be evaluated by directly comparing population genetics to landscape variables that either interrupt or facilitate gene flow. Here, we evaluate genetic connectivity between populations to identify patterns that might signal some degree of population isolation related to landscape factors. Based on classic population genetics analyses (Nadolny at al. 2015, Benham et al. 2021), Φ_{ST} values predict that populations are not well-connected, but long-distance dispersal events can occur. Dispersal of GCT is thought to be the primary means of introducing *R. parkeri* into novel environments.

Materials and Methods

Sampling

Sample sites were determined through county-by-county sweeps across Virginia to identify sites with Gulf Coast tick presence. Sampling continued bi-weekly at regularly sampled GCT sites (Benham et al. 2021), and at sites throughout the eastern Virginia coastal plain, focusing specifically where GCT had been collected in prior years to identify any new populations. Overall, 980 GCT have been collected between 2017-2020 by the ODU Tick Team using both regular flagging and special sampling to increase GCT collections.

Genetics

GCT samples were processed using a protocol described in Benham et al. (2021) to isolate target mitochondrial DNA

fragments, amplify fragments using polymerase chain reaction (PCR), and sequence 217-218 nucleotide base pairs by Sanger Sequencing. Forward and reverse sequence reads were aligned in Geneious (Kearse et al. 2012).

To prepare a genetic dataset for the Population Graph analyses, finalized tick haplotypes from 2017-2019 ticks were filtered to include only those haplotypes that had no ambiguities, no evidence of heteroplasmy, and at least 4 haplotyped individuals per sampling location (hereafter referred to as "populations" or "nodes"). The total number of ticks included in the Population Graph analysis were 287, subsequently filtered to 280 after removing a subset of ticks from Louisiana because precise geographic coordinates were not available for these individuals.

Analysis

A Population Graph for GCT populations was created using the R package 'popgraph' (Dyer 2014). A Population Graph consists of nodes and edges that create a network showing connectivity between populations based on genetic covariance. Nodes are the populations studied and edges are derived from the genetic covariances between populations. Edge weight denotes the genetic distances between populations. Three features of the population graph are considered here: saturation, degree, and conditional graph distance (cGD). Population Graphs are considered saturated when all of the nodes are connected to one another and nonsaturated when at least one edge is missing such that at least one pair of nodes are not connected. Nonsaturated Population Graphs provide an opportunity to explore the underlying causes of interrupted gene flow. Degree is a measure of the number of edges connecting to a node.
Conditional graph distance is a type of correlation test using Spearman's rho that can be used to identify cases of isolation-by-distance by comparing spatial distance between populations to the genetic covariances (Dyer et al. 2010).

Results

All of the GCT populations (n = 6) evaluated in this study were connected to at least 3 other populations, with degree ranging from 3 to 5. As some nodes were not connected to one another, the population Graph was nonsaturated. The site BI3 had the smallest observed edge set (e = 3). Louisiana samples had the largest number of edges connecting nodes (e = 5). However, the Louisiana samples were removed from further analyses.

Table 1. Edge weights from the GCT Population Graph based on genetic covariances among 280 individuals from 6 populations.

	1	2	3	4	5	6
1	0.00	-	-	-	-	-
2	2.35	0.00	-	-	-	-
3	0.00	1.78	0.00	-	-	-
4	2.76	0.00	2.18	0.00	-	-
5	4.68	4.61	4.19	4.41	0.00	4.28
6	0.00	1.88	1.36	2.27	4.28	0.00

We found no evidence of isolation by distance for the Virginia and North Carolina populations (n= 6, Spearman's rho= 0.005, p =0.84).

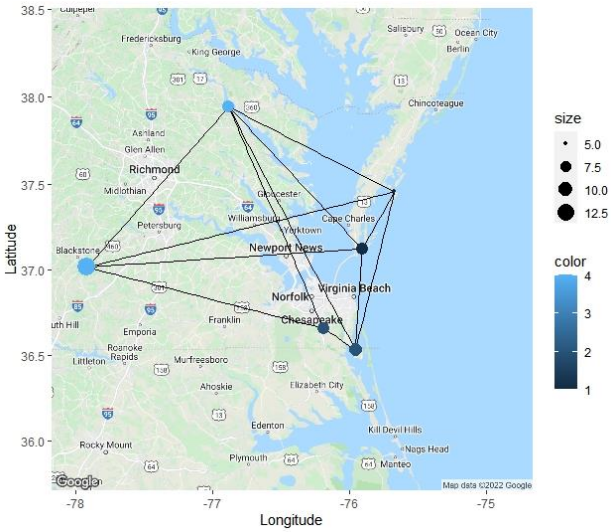

Figure 1. Population graph overlying spatial coordinates for each sampling location in Virginia and North Carolina. Node size is the genetic covariance within each population. Colors denote population groupings as barrier island, coastal, or inland.

Discussion

GCT populations in the mid-Atlantic United States appear to have a high degree of connectivity based on analysis of 16S mitochondrial haplotypes. Here, we used a Population Graph approach that maps connectivity more explicitly than Φ_{ST} values used in prior studies. Further, this study compares additional samples from 2019, which were not previously analyzed (Nadolny et al. 2015, Benham et al. 2021). The Population Graph shows evidence of gene flow to each population from multiple sources, without apparent directionality. From this, we conclude that GCT populations are panmictic. In other words, dispersal from one population to another and interbreeding between populations occurs frequently.

Despite evidence of geographic structuring of *R. parkeri* prevalence in GCT, population genetics analyses of GCT have yet to clarify underlying patterns of gene flow. Several additional steps can help to elucidate this question. First, inclusion of more populations is essential to improve our ability to analyze geographic patterns (Dyer 2015). Identifying suitable habitat for GCT and collecting individuals across a wide geographic area can be time and labor intensive. GCT populations are often ephemeral, which makes year-to-year monitoring difficult. The transient nature of these populations is likely due, at least in part, to the close association of GCT with recently-disturbed habitat followed by rapid population decline when habitat transitions to later successional stages (Nadolny and Gaff 2018). New reports of GCT populations established in Arizona, Connecticut, and Illinois provide an opportunity to expand surveillance of both GCT and *R. parkeri*.

The next step in this project is to evaluate the topological congruence between GCT and *R. parkeri* Population Graphs to determine whether *R. parkeri* diversity and connectivity patterns deviate from that which has been observed in GCT. This is an essential step to identify the geographic link between these symbiotic taxa. For example, if *R. parkeri* populations exhibit similar connectivity patterns, then it is likely that the dispersal of ticks is also contributing to the spread of the pathogen at a roughly similar rate. Alternatively, *R. parkeri* prevalence could be driven by the sylvatic cycle, i.e. infection of wildlife hosts, after the pathogen is initially introduced. In that scenario, ticks might still contribute to the introduction of *R. parkeri* into a new ecosystem, but high prevalence in local ticks might reflect amplification of *R. parkeri* in novel hosts rather than indicating that ticks are immigrating from source populations with high levels of infection. Landscape genetics simulations can help to develop the theoretical basis for additional hypothesis testing based on current knowledge of host, tick, and pathogen biology.

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